

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWERS APPLY.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept
responsibility for the scientific conduct of the project and to provide the required
progress reports if a grant is awarded as a result of this application. Willful provi-
sion of false information is a criminal offense. (U.S. Code, Title 18, Section 1001.)

15, CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)

SIGNATURE OF PERSON NAMED IN 2a (In ink. "Per" signature not acceptable)

Wendell F. McBurney 317

Stourffe pwon SIGNATURE OF PERSON NAMED IN 12c (In ink. Per" signature not acceptable)

DATE DATE

274-8285

KWON00056

NEXT BUDGE	JDGET FOR FROM			ROUGH		NUMBER	
Follow instruction					AI	28175-2	
A. ITEMIZE DIRECT COSTS RE	QUESTED FOR NEXT BUDGET PERIC	D 1	2	3	DOLLAR AM	OUNT REQUEST!	ED (Omit ce
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NAME	ROLE IN PROJECT	APPT.	APPT.	PROJ.	SALARY	BENEFITS	TOTAL
Byoung S. Kwon	Principal Investigator	1.0	46	0.46	28,130	9,522	37,65
_ Karen Z. Pollok	Postdoctoral Fellow	1.0	100	1.0	20,000	4,980	24,980
Hal E. Broxmeye	r Co-Investigator	1.0	3	0.03	2,388	808	3,19
Kwi 0. Oh	Co-Investigator	1.0	100	1.0	13,532	3,370	16,90
Yvonne Kobayash	i Research Tech.	1.0	100	1.0	20,168	5,023	25,19
71 71	Visiting	1.0	30	0.3	4,400	1,096	5,49
Zhen Zhou	Scientist	1.0	30	0.3	4,400	1,090	7,45
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And the second s	SUBTOTALS				88.618	24,799	113,41
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GRANT NUMBER

SECTION I (continued) NEXT BUDGET PERIOD

AI 28175-02

B. SUPPLEMENTAL INFORMATION REGARDING *ITEMS* IN THE PROPOSED BUDGET FOR THE NEXT PERIOD WHICH REQUIRE EXPLANATION OR JUSTIFICATION. (SEE INSTRUCTIONS)

Personnel

<u>Dr. Byoung S. Kwon</u>, Principal Investigator (46% effort), is responsible for the overall conduct of this research. His efforts include performance of daily work at the laboratory bench; coordination of work with the collaborating laboratories of Drs. Hal Broxmeyer, Leonard Schultz, Sherman Weissman, and John Ding-E Young; administration of the project budget, and writing of annual reports. Dr. Kwon will design and construct expression plasmids, and perform receptor-binding studies and functional assays, evaluating each experimental step in the program.

<u>Dr. Karen Pollok</u>, (100% effort) is a Postdoctoral fellow in this laboratory. Her responsibilities will include protein purification of soluble 4-lBB and perforin and a variety of immunological assays. Dr. Pollok will perform a number of biochemical and receptor-binding assays to characterize 4-lBB molecule.

<u>Dr. Hal Broxmeyer</u>, Coinvestigator (3% effort), participates in evaluating functions of recombinant products of L2G25B and L2G25C in both in vitro and in vivo.

Dr. Kwi-Ok Oh, Coinvestigator (100%), a Visiting Scientist, has been receiving training in this laboratory since. She will maintain cell culture, transfect recombinant DNA and perform protein purification and receptor-binding assays L2G25B and L2G25C proteins under the instruction of Dr. Kwon.

Miss Yvonne Kobayashi, (100%) Research Technician, will prepare DNAs, perform Southern, Northern, and Western blotting procedures, and screen hybridomas by dotimmunoassay. Yvonne is well-trained in DNA-sequencing. She is also well trained in handling vaculovirus expression systems. She has been producing recombinant proteins for L2G25C, perforin and 4-1BB. Yvonne will also be involved in purification of a large quantity of perforin to crystalize.

 $\underline{\text{Dr. Zhen Zhou}}$, (30%) Visiting Scientist, will perform immunocytochemistry to localize the 4-1BB proteins in various mouse organs. He is also doing $\underline{\text{in situ}}$ hybridization with the gene probes we generated. Dr. Zhou will also be involved in the studies on 4-1BB and perforin expression in the T cells infiltrated in autoimmune insulitis and arthritis of mice.

Consultants \$600

The amount of \$600 is requested to defray travel expenses of Dr. Kwon for the purpose of conferring once each year with Drs. Weissman and Shultz. No cost-increase is projected in subsequent grant-years for this consultant expense.

<u>Dr. Sherman</u> Weissman will continue to provide his expertise in nucleic acid research in all phases of this project. He advises on production of recombinant proteins in both prokaryotic and eukaryotic expression systems. He also evaluates the work of this laboratory as to scientific merit.

<u>Dr. John D.-E Young</u> consults with Dr. Kwon on protein purification and assays on CTL activity.

<u>Dr. Leonard Shultz</u>, of Jackson Laboratories, provides immunodeficient mutants collected by him and also provides new mutants as they arise.

 $\underline{\text{Dr. Stephen Litwin}}$ consults with this laboratory on B-cell maturation, immunoglobulin production, and assays on B-cell functions.

<u>Dr. Himadri Samanta</u> provides his expertise on the production of recombinant DNAs using the bovine papilloma viral expression system. He also advises on the technologies for purification of the recombinant proteins.

KWON00058

<u>Supplies</u> \$23,280

Items in the supply budget remain the same as in grant-year 2, and a 6% increment is applied to allow for increases in costs.

Travel \$1,200

Dr. Kwon and Dr. Pollok plan to attend FASEB meeting.

Other Expenses \$1,600

Costs of publication (page charges, artwork, etc.), computer costs, and mailing/long-distance telephone expenses remain the same as in year 2.

SECTION II	FROM	THROUGH	GRANT NUMBER	
CURRENT BUDGET PERIOD				
AND KEY PERSONNEL			AI 28175-02	

The following pertains to your CURRENT PHS budget. This information will be used in determining the amount of support for the NEXT budget period.

A. CURRENT BUDGET	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (1)	ESTIMATED UNOBLIGATED BALANCE (2)	EXPLAIN ANY SIGNIFICANT ESTIMATED UNOBLIGATED BALANCE IN COLUMN 2 (3)
TOTAL DIRECT COSTS	99,493	99,493	
INDIRECT COSTS (As provided)	48,105	48,105	
TOTALS>	147,598	147,598	

B. CURRENT BUDGET PERIOD KEY PERSONNEL ENGAGED ON PROJECT (Only if different)

NAME, DEGREE(S) SSN	POSITION TITLE AND ROLE IN PROJECT DEPARTMENT AND ORGANIZATION	CHANGE IN % OF EFFORT
Karen Z. Pollok, Ph.D. 231-84-3958	Postdoctoral Fellow	+ 1.0
Yvonne Kobayashi, B.S. 306-64-6558	Lab Technician	+ 1.0
Zhen Zhou, M.D.	Visiting Scientist	+ 0.3

C. and D. (Only if different)

See instructions and provide the information required in Items C. and D. Use this page and continuation pages as necessary.

See Attached

SECTION III. PROPOSED KEY PERSONNEL FOR THE NEXT BUDGET PERIOD (Only if different)

NAME, DEGREE(S), SSN	POSITION TITLE AND ROLE IN PROJECT	DEPARTMENT AND ORGANIZATION
Karen Z. Pollok, Ph.D.	Postdoctoral Fellow	Microbiology and Immunology Indiana University School of Medicine
Yvonne Kobayashi, B.S.	Lab Technician	Microbiology and Immunology Indiana University School of Medicine
Zhen Zhou, M.D.	Visiting Scientist	Microbiology and Immunology Indiana University School of Medicine
		KWON00060

C and D (continued)

- C. Equipment Other funds were identified to purchase the requested Micro centrifuge. No other equipment was purchased.
- D. <u>Travel</u> The following trips were supported:
 - 1) B.S. Kwon to Berlin, W. Germany 7th International Congress of Immunology \$1,242 Presented a poster.
 - 2) B.S. Kwon New Orleans, LA \$727 ASBMB/AAI Joint Meeting - Spoke at a mini-symposium and presented a poster.
 - 3) Kack Kim Hilton Head, SC \$460
 Second International Workshop on cytokines.

 Presented a poster. Dr. Kim's salary is not supported by this grant but he is studying perforin gene expression, a part of this project. Dr. Kim is Dr. Oh's husband.
 - 4) Dr. Oh Hilton Head, SC \$257
 Second International Workshop on cytokines.

 Presented a poster.

OTHER SUPPORT

(Use continuation pages if necessary)

GRANT NUMBER

AI 28175-02

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to delays in the award. OTHER SUPPORT to be listed here refers to all current or requested support whether related to this application or not. If there are changes subsequent to submission, notify the Grants Management Official named on the Notice of Grant Award.

For each of the key personnel named on page 4, list, in three separate groups: (1) all currently active support; (2) all applications and proposals pending review or funding; and (3) applications and proposals planned or being prepared for submission. Include all Federal, non-Federal (e.g., for-profit, pharmaceutical, foundations), and institutional research, training, and other grant, contract, and fellowship support at the applicant organization and elsewhere. If part of a larger project, identify the principal investigator/program director and provide the data for both the parent project and the subproject. If none, state "none."

For each item give: (a) the source of support, identifying number and title; (b) percentage of appointment on the project; (c) dates of entire project period; (d) annual direct costs; (e) a brief description of the project; (f) whether the item overlaps, duplicates, or is being replaced or supplemented by the present application; delineate and justify the nature and extent of any scientific and/or budgetary overlaps or boundaries; and (g) any modifications that will be made should this continuation award be made.

PRINCIPAL	INVESTIGATOR/PROGRAM DIRECTOR:

- (1) CURRENTLY ACTIVE SUPPORT: (a)
 - a) NIH Grant 1R01 AR40248-01; Melanin Biosynthesis and Oculocutaneous Albinism
 - b) B.S. Kwon, Principal Investigator. 10% effort. c)
 - d) Annual cost \$128,705 e) Studies on the molecules involved in melanin pigment synthesis. f) Not overlap with current project.
 - a) Schering-Plough Corporation; Regulation of Melanin Biosynthesis (Chemicals)
 - b)B.S. Kwon, Principal Investigator. 10% effort. c)
 - d) Annual cost \$140,500 e) Induction of darkening and lightening of skin pigment by chemical agents. f)

 Not overlap with current project.
 - a) March of Dimes Birth Defects Foundation; Molecular Basis of tyrosinase-negative albinism b) B.S. Kwon, Principal Investigator. 5% effort. c)
 - d) Annual direct cost \$35,000 e) Molecular genetic studies on the mutation responsible for tyrosinase-negative albinism. f) Not overlap with current project.

KWON00062

SECTION IV	GRANT NUMBER		
PROGRESS REPORT SUMMARY	AI 28175-	-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR	PERIOD CO	OVERED BY THIS REPORT	
Byoung S. Kwon, Ph.D.	FROM	THROUGH	
APPLICANT ORGANIZATION	***************************************	***************************************	
Indiana University School of Medicine			
TITLE OF PROJECT (Repeat title shown in item 1 on first page)			
Characterization of three new T lymphocyte-spe	ecific gene		

1. The main objective for the forthcoming project year is to determine the functions of L2G25B, L2G25C (formerly called L2G95B) and 4-1BB. We will also study the domain-specific function of perforin. We first prepared antibodies against oligopeptides representing the deduced primary sequence of each molecule and then prepared recombinant proteins of these molecules. The following is a brief summary of unpublished results and future plans.

A. 4-1BB:

(SEE INSTRUCTIONS)

4-1BB protein is a receptor molecule which is inducible in T cells and is expressed constitutively in neurons and in certain medulla cells in kidney. Cytoplasmic domain of 4-1BB contains consensus sequence (-C-R-C-P-) for the binding to p561ck, a protein tyrosine kinase. We are preparing soluble form of the 4-1BB, which is recombinant protein without the transmembrane domain. This can be a specific inhibitor to the 4-1BB, as shown in other lymphokine receptors. We have expressed 4-1BB in C127 cells using bovine papilloma viral vector and in insect cells (sf-9) using baculoviral vector. These material will be used for two purposes; 1) to find the potential ligand; and 2) to determine whether 4-1BB transduces signal through binding to protein tyrosine kinases such as p561ck.

B. Perforin:

Perforin is believed to be a key molecule in killing cells bearing foreign antigens by forming a transmembrane channel. We isolated and characterized the cDNA and a gene for mouse perforin. We have been successful in producing the recombinant perforin (both human and mouse) in a baculoviral expression system. The purpose is to crystalize the molecule to understand its three dimensional structure. This information in turn will be used to design experiment to determine the domain-specific functions of this molecule.

C. L2G25B and L2G25C:

We have prepared a large quantity of recombinant proteins for both genes. According to in vitro experiments done in collaboration with Dr. Hal Broxmeyer the L2G25B and L2G25C recombinant proteins have direct myelopoietic enhancing activity for mature progenitors, while L2G25B, but not L2G25C, has direct suppressing activity for more immature progenitors. We plan to determine the physiological relevance of the myelopoietic enhancing and suppressing activities of these molecules in animal models. We are also seeking cells bearing receptors to the L2G25B and L2G25C proteins. The cells that we are employing at present are FDCP-Mix, 416B, and DU528, which represent bone marrow stem cell lines; I1-1-activated endothelial cells; activated spleen cells; and primary bone marrow cell mixture. We hope this study leads to finding the target cells through which we can study in depth the functions of these small molecules.

- 2. Our main effort was directed to prepare reliable reagents to utilize for the determination of functions of T-cell molecules, 4-1BB, L2G25B, L2G25C and perforin. All the molecules have been produced in recombinant form and antibodies recognizing the natural molecules are prepared. In addition to the preparative work mentioned above, we found followings in each molecule. 1) 4-1BB was expressed not only in T-cells but also in neuron and kidney cells. 4-1BB was expressed in early stage of insulitis but not in later stage of insulitis in NOD mice. 2) 5' UTR (untranslated region) of mouse perforin is formed by alternative splicing. The perforin mRNA was degraded within 5 min. when the killer cells (CTL, NK and LAK) made a full contact with specific target. Mouse perforin gene was characterized and mapped to chromosome #10. 3) L2G25B and L2G25C recombinant proteins have direct myelopoietic enhancing activity for mature progenitors, while L2G25B, but not L2G25C, has direct suppressing activity for more immature progenitors. Our recombinant proteins for L2G25B and L2G25C were as active as natural ones.
- 3. and 4. Non-applicable.
- 5. Seven papers have been published or are now in-press and one is in submission during the second grant-year: Copies of those papers are attached.
- Kwon, B.S., Kestler, D.P., Eshhar, Z., Oh, K.-O. and Wakulchik, M. Expression characteristics of two potential T cell mediator genes. <u>Cellular Immunology</u> 121: 414-422, 1989.
- Qureshi, M., Yoon, J.W. and Kwon, B.S. Identification and production of monoclonal antibodies against a discriminating protein molecule between B and D variants of encephalomyocarditis virus. Develop. Comp. Immunol. 13: 263-271, 1989.
- Liu, C.C., Joag, S.V., Kwon, B.S., and Young, J.D-E. Induction of perforin and serine esterases in murine cytotoxic T lymphocyte clone. <u>J. Immunol</u>. 144: 1196-1201, 1990.
- Joag, S.V., Liu, C.C., Kwon, B.S., Duke, R.C., Clark, W.R., and Young, J.D.-E. The expression of pore-forming protein and two serine esterase genes in murine primary and cloned effector lymphocytes. <u>J. Cell. Biochem</u>. 43: 81-88, 1990.
- Trapani, J.A., Kwon, B.S., Kozak, C.A., Chintamaneni, C., Young, J. D-Z and Dupont, B. Genomic organization of the mouse pore-forming protein (perforin) gene and localization to chromosome 10. \underline{J} . $\underline{\text{Exp}}$. $\underline{\text{Med}}$. 171:545-557, 1990.
- Young, J.D.-E., Kwon, B.S., Trapani, J.A., Liu, C.-C. and Young, L.H. Lymphocyte-mediated cytolysis: role of granule mediators. <u>Subcellular Biochem</u>. (in press).
- Broxmeyer, H.E., Sherry, B., Cooper, S., Oh, K., Tekamp-Olson, P., Kwon, B.S., and Cerami, A. Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation $\underline{\text{in vitro}}$ by bone marrow myeloid progenitor cells. $\underline{\text{Blood}}$. (in press).
- Bajapi, A., Kwon, B.S. and Brahmi, Z. Rapid loss of perforin and serine protease RNAs in cytotoxic lymphocytes exposed to sensitive targets. J. Immunol. (submitted)

	GRANT NUMBER		
CHECKLIST	AI 28175-02		
Check the appropriate boxes and provide the information requested. Mal Do not attach copies of this page to the duplicated copies of the application	ke this page the last page of the signed original of the applicat		
ASSURANCES			
The following certifications described below are made by checking the appr SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the app	ropriate boxes and verified by the signature of the OFFICIAL lication.		
a. Delinquent Federal Debt. X No Yes (If "Yes," attach exp Before a grant award can be made, the applicant organization must certification applies to the applicant organization, not to the person principal investigator/program director.	fy that it is not delinquent on the renayment of any Federal debt		
Examples of Federal debt include delinquent taxes, audit disallowances, other miscellaneous administrative debts. For purposes of this certificate	guaranteed or direct student loans, FHA loans, business loans, a tion, the following definitions of "delinquency" apply:		
For direct loans and fellowships (whether awarded directly to the app funds), a debt more than 31 days past due on a scheduled payment. Service Award.)	olicant by the Federal Government or by an institution using Fede (Definition excludes "service" payback under a National Resea		
For guaranteed and insured loans, recipients of a loan guaranteed by repurchased from a lender because the borrower breached the loan a	agreement and is in default.		
For grants, organizations in receipt of a "Notice of Grants Cost Disallhave not resolved the disallowance. (Definition excludes disallowance)	ces in an "appeal" status.)		
Where the applicant discloses delinquency on debt to the Federal Gover determining whether the prospective grantee organization is responsible until payment is made or satisfactory arrangements are made with the ag for the PHS to contact the applicant before a grant can be made to confi for its liquidation. Applicants that fail to liquidate indebtedness to the Fe risk of not receiving financial assistance from the PHS.	with respect to that grant, and (2) consider not making the grant gency to whom the debt is owed. Therefore, it may be necessary in the status of the debt and ascertain the payment arrangement.		
b. Debarment and Suspension. X No Yes (If "Yes," attach			
Before a grant award can be made, the applicant organization must certif debarred, suspended, proposed for debarment, declared ineligible, or vol department or agency. Subawardees, that is, other corporations, partner make the same certification to the applicant organization concerning their implementing regulations, Title 45 Code of Federal Regulations Part 76, for	luntarily excluded from covered transactions by any Federal erships, or other legal entities (called "lower tier" participants), m r covered transactions. Please refer to the perticent DHHS		
c. Drug-Free Workplace. X Yes No (If "No," attach explan	·		
Before a grant award can be made, the applicant organization must certification require the applicant organization to:			
Publish a statement notifying employees that the unlawful manufacture substance is prohibited in the workplace and specifying the actions the state of the state	e, distribution, dispensation, possession, or use of a controlled hat will be taken against employees for violation of such prohibiti		
Establish a drug-free awareness program;Require that each employee engaged in the performance of a grant or	r contract he provided a copy of the published statement		
Notify the employee that as a condition of employment, the employee	will abide by the terms of the statement;		
Notify the PHS awarding component of any employee convicted of a			
Require any employee who is convicted of a drug offense occurring in Please refer to the pertinent DHHS implementing regulations, Title 45 Cod requirements.	n the workplace to participate in a renabilitation program. de of Federal Regulations Part 76, for complete certification		
INDERECT COST CALCULATION Indicate the applicant organization's most recent indirect cost rate established for profit organizations, the rate established with the appropriate PHS Agencies.	v Cost Advisory Office Indirect costs will not be paid on forci		
grants, construction grants, grants to Federal organizations and grants to inc additional instructions provided for Research Career Development Awards, Ir grant applications.	dividuals, and usually not on conference grants. Follow any nstitutional National Research Service Awards, and specialized		
X DHHS Agreement Dated:	No Indirect Costs Requested		
No DHHS Agreement, but rates established with	DATE		
*CALCULATION Enter proposed budget period:			
	% = Indirect Costs \$68,647		
Add to total direct costs from page 2 and ente *Check appropriate box(es)	er new total on FACE PAGE, Item 10b		
Salary and wage base X Modified total direct cost	sts base Other base (Attach explanation)		
Off-site, other special rate, or more than one rate involved (Attach ex			
1 1 - 1 one, one, openin rate, or more than one rate myorven (Attach e)	KWON000		

*This is the required last page of the application.